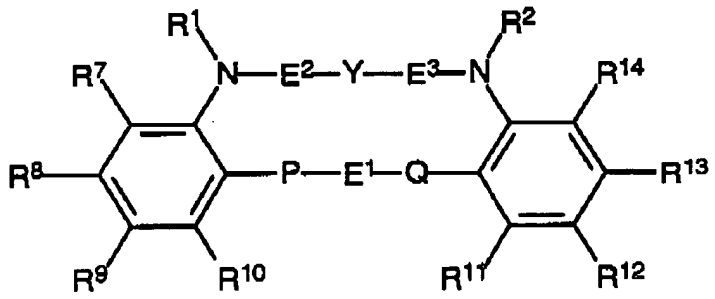
REMARKS

The Claimed Invention

The present invention is directed to derivatives of crown ether compounds that are required to comprise a dye, a reactive group or a conjugated substance. These crown ether compounds have the following general structure:



wherein the substituents are as defined in the claims. The present crown ether compounds bind sodium, calcium and potassium ions under physiological conditions and when attached to a fluorophore are fluorogenic ion indicators. Thus, these crown ether compounds find particular use as indicators of said metal ions wherein they demonstrate a changed fluorescent signal when bound to ions such as sodium.

The Pending Claims

Prior to consideration of the following Response to Office Action, Claims 1-51 are pending. Claims 1-20 are directed to the present crown ether compounds comprising at least one –L-DYE, -L-Rx or –L-Sc. Claims 21-43 are directed to the present crown ether compounds wherein the compounds comprise at least one –L-DYE. Claims 44-50 are directed to a method for detecting target metal ions in a sample using the present crown ether compounds. Claim 51 is directed to a kit for detecting or quantitating target metal ions.

The Office Action

Claims 21-41 and 44-51 are withdrawn from consideration.

Claims 1-21 are rejected under a judicially created doctrine as being drawn to an improper Markush group.

Claims 1-21, 42 and 43 are rejected under 35 U.S.C. 112, 2nd Paragraph for containing indefinite claim language.

Amendments

Claims 1, 3, 5, 7, 10, 12, 14-16, 18-19, 21, 25, 28-30, 34-35, 38, 42, 43, 44 and 51 have been amended.

Claim 1 has been amended to clarify the reactive group Markush group as recommended by the Examiner. The language "selected from the group consisting of" has been added.

Claims 1, 21, 42 and 44 have been amended to replace "biologically compatible esterifying group" with "alpha-acyloxyalkyl and t-butyldimethylsilyl". Support can be found in Claims 20, 39, 43, 48 and 51 as originally filed and on page 5 line 5 of the specification.

Claim 2 has been cancelled.

Claim 3 has been amended to depended from newly added Claim 52.

Claim 4 has been cancelled.

Claim 5 has been amended to depend from newly added Claim 52.

Claim 7 has been amended to depend from Claim 5.

Claim 10 has been amended to depend from Claim 7.

Claim 11 has been cancelled.

Claim 12 has been amended to depend from newly added Claim 54.

Claim 13 has been cancelled.

Claim 14 has been amended to depend from Claim 6.

Claim 14 has been amended to add "synthetic polymer". Support can be found in Claim13 as originally filed.

Claim 15 has been amended to depend from Claim 6.

Claim 16 has been amended from dependent form to independent form.

Claim 16 has been amended to include the limitations of Y, R^1, R^2, R^7, R^8 and R^{10}-R^{14} as found in originally filed Claim 1.

Claims16 and 21 have been amended to include the reactive group limitations found in Claim 1.

Claim 16 has been amended to include specific conjugated substances (Sc). Support can be found in Claim 13 as originally filed and on page 30 lines 23-31.

Claim 18 has been amended to replace "DYE" with "xanthene" and to clarify Markush group. Support can be found in Claim 17 as originally filed.

Claim 19 has been amended to depend from Claim 18.

Claims 22-24 have been cancelled.

Claim 25 has been amended to depend from newly added Claim 55.

Claim 28 has been amended to depend from Claim 27.

Claims 29 and 30 have been amended to depend from Claim 25.

Claim 33 has been cancelled.

Claims 34 and 35 have been amended to depend from Claim 26.

Claim 38 has been amended to depend from Claim 37.

Claims 43, 48 and 51 have been amended to correct a typographical error in the spelling of alkoxy.

Support for new Claims 52-55 can be found in the claims as filed and throughout the specification. In particular, support for Claim 52 and 55 can be found in Claims 1, 16, 21 and 36. Support for new Claim 53 can be found in Claim 1. Support for Claim 54 can be found in Claim 17 as originally filed. Support for DYE moiety language can be found in Claims 8, 17 and 18 as originally filed and on page 15 lines 24-31, page 16 lines 1-7 and page 17 lines 5-7.

Applicants believe that no new matter has been added by any of these amendments and the Examiner is respectfully requested to enter them.

Martin *et al.*
Serial No. 10/026,302

RESPONSE TO THE ELECTION/RESTRICTION REQUIREMENT

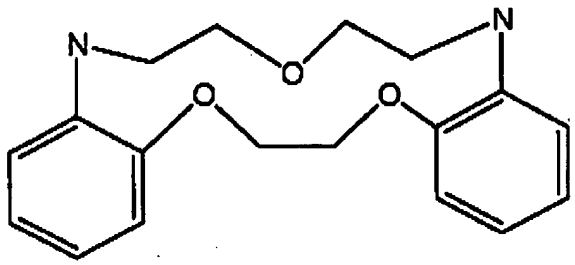
In the response that follows, the Examiner's Election/Restriction of the Applicant's claimed invention is provided in full text, as identified by indented small bold print, followed by the Applicants response.

Applicant's election of compound 138 on pages 56-57 in Paper No. 10 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP 818.03(a)).

Applicants respectfully point out that there was no restriction requirement made by the Examiner in Paper No. 9, only an election requirement. MPEP 803.1 states that when issuing a restriction requirement "Examiners must provide reasons and or reasons to support conclusions". The Examiner did not split the claims up into groups based on what he believed to be the supposed separate inventions or provide any statement as to why supposedly there is more than one invention present in the current claims set. Thus, there was nothing for the Applicant to "distinct and specifically point out to be the supposed errors in the restriction requirement" as there was no restriction requirement.

As required by 35 U.S.C. 121 Applicants elected a species for the *purposes of initiating a search and examination*. Thus, a species election was made without traverse but applicants in no way intended to elect a group of claims without traverse.

The elected compound was not found in the search and is allowable. The search was expanded to embrace the core



Thus, compounds of this scope of the core identified above along with the definitions of the variables have been examined.

Claims 21-41 and 44-51 are withdrawn from consideration because they are drawn to methods and kits that raise different issues of patentability and require separate searches.

Applicants respectfully request the Examiner to provide reasoning as to why he believes the method and kit claims are patentably distinct from the compound claims and from the method claims, as none has been provided, demonstrating that the inventions are independent or distinct as claimed and that there is a serious burden on the Examiner to perform search and Examination of the current claims 1-51. In addition, Claims 21-41 are not drawn to methods or kits but to *compositions*. As such, Applicants do not believe a proper restriction requirement has been made and object to Claims 21-41 and 44-51 being withdrawn. Applicants respectfully request that these claims be rejoined with Claims 1-20 or provide adequate reasoning as to why the withdrawn claims are independent inventions.

RESPONSE TO THE REJECTIONS

In the response that follows, the Examiner's individual rejections of the Applicant's claimed invention is provided in full text, as identified by indented small bold print, followed by the Applicants response.

Improper Markush Relection

Claims 1-21 are rejected under judicially created doctrine as being drawn to an improper Markush group, that is, the claims lack unity of invention. The variable E^1, E^2, E^3, Y, P and Q are defined in such a way that they keep changing the core of the compound that determines the classification. By changing these values, several patentably distinct and independent compounds are claimed. In order to have unity of invention the compounds must have "a community of chemical or physical characteristics" which justify their inclusion in a common group, and that such inclusion is not repugnant to principles of scientific classification" In re JONES (CCPA) 74 USPQ 149. The structural formula of the compound of Claim 1 does not have a significant structural feature that is shared by all of its alternatives which is inventive. The structure has only two benzene rings as common. This feature is not inventive. Compounds embraced by these claims are so diverse in nature that a prior art anticipating a claim with respect to one member under 35 USC 102 would not render obvious the same claim under 35 USC 103. This is evidentiary of patentably distinct and independent inventions

Limiting the claims to the group identified above as searched would overcome this rejection.

Applicants respectively traverse this rejection because there is a unity of invention and the Markush group is proper.

The crown ether compounds of the present invention contain two aryl groups and two directly substituted nitrogen atoms that form a ring structure. On the bottom part of the ring (See structure above), the aryl rings are separated by at least two heteroatoms (O, S or NR^3) and an alkyl group that contains 2 to 4 carbon atoms or $-(C(O)CH_2)$ that contains 4-8 carbon atoms. The nitrogen atoms on the top part of the crown structure are separated by at least one heteroatom (O, S, or NR4) and independently two alkyl group that contains 2 to 4 carbon atoms or $-(C(O)CH_2)$ that contains 4-8 carbon atoms.

Thus, E1, E2 and E3 are independently and alkyl group that contains 2 to 4 carbon atoms or $-(C(O)CH_2)$ that contains 4-8 carbon atoms and Y, P and Q are independently a heteroatom selected from the group of O, S, or NR^3 or NR^4. These substituents can be selected in a way that results in many different compounds of the present invention. However the requirement of the aryl rings, nitrogen atoms and their substituents R^1 and R^2 provide novelty and as such the alternatively compounds are not independent inventions. The present inventions envisions many different compounds wherein the core structure remains constant and when covalently attached to a DYE moiety they function as chromogenic or fluorogenic metal ion indicators.

Therefore, Applicants respectfully request that the rejection of Claims 1 and 21 be withdrawn.

Applicants have added new claims 52 and 55 that depend from claims 1 and 21 wherein subsequent dependent claims depend from these claims. E^1, E^2, E^3, P and Q have been limited to $-(CH_2)_2-$ and O respectively. Y is still permitted to be O, S or NR^4. Claim 5 further limits Y to O wherein all subsequent dependent claims, excluding Claim 3 wherein Y is NR^4, contain this limitation. Thus, Applicants respectfully request that this rejection of Claims 2-20 be withdrawn in view of newly added Claims 52 and 55.

In addition, Applicants respectfully point out that Examiner has both withdrawn and rejected Claim 21.

35 U.S.C. 112, 2nd ¶ Rejection

Claims 1-21, 42 and 43 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

This rejection is respectfully traversed because the following terms are properly defined in the specification. These claim terms must be examined in view of 1) the content of the present application, 2) the teachings of the prior art, and 3) the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made (MPEP 2173.02). The Applicants assert that, in view of these criteria, the claim terms "heteroaryl", "DYE", "a biological compatible esterifying group", "a biological compatible salt", "Rx" and "conjugated substance" are properly defined in the present application.

In addition, Applicants respectfully point out that Examiner has both withdrawn and rejected Claim 21.

i) **The term "heteroaryl" is indefinite because it is not known how many atoms are present, how many and what kind of heteroatoms are involved, what size ring is intended and how many rings are present.**

Applicants respectfully traverse this rejection because the specification clearly defines the limitations of heteroaryl. The specification states "for the purposes of this invention, a heteroaryl ...means a 5- or 6-membered unsaturated ring that is optionally fused to an additional six-membered aromatic ring(s), or is fused to a 5- or 6-membered unsaturated ring containing one or more heteroatoms...each ring contains at least 1 and as many as 3 heteroatoms that are selected from the group consisting of O, N or S in any combination" (page 6 lines 16-21). This is followed by specific examples of heteroaryl ring systems (page 6 lines 21-30).

Therefore, the present specification provides guidance as to the meaning of the claim term "heteroaryl" wherein the number of atoms that are present, the number and kind of heteroatoms involved and the intended ring size and how many are defined. Thus, based on the claim language and the teaching of the present specification Applicants respectfully assert that "heteroaryl" is not indefinite and request that the Examiner withdraw this rejection of Claims 1-21, 42 and 43.

ii) **The group "DYE" is defined as "a chemical moiety with an absorption maximum beyond 320 nm. One of skill in the art cannot say which chemical moiety has an absorption maximum beyond without taking the billions of chemical moieties known and testing each. Thus, this group is open-ended.**

Applicants respectfully traverse this rejection because the specification properly defines the term "DYE". The specification defines the term "DYE" functionally as "any chemical moiety that exhibits an absorption maximum beyond 320 nm, that is bound to the crown chelate by a covalent linkage L, or is fused to the crown chelate" (page 14 lines 8-10). The specification further limits this definition by stating "the dye moiety may be a chromophore, resulting in a compound that acts as a chromogenic indicator, or more preferably, DYE is additionally a fluorophore, resulting in a compound that is a fluorescent indicator" (page 14 lines 24-29). Thus, the specification functionally defines "DYE" and then further defines the DYE to be a chromophore or fluorophore.

Based on this definition it is clear to one of skill in the art what is a DYE. However the specification further teaches that the DYE moiety typically contains one or more aromatic or heteroaromatic rings that are optionally substituted one or more times by a variety of substituents (page 14 lines 17-18). Thus, the chromophores or fluorophores that are the DYE moiety typically contain an aromatic ring, yet another distinguishing feature that provides limits of the DYE moiety. Furthermore, the specification provides examples of known DYE moieties (page 15 lines 14-31:page 16 lines 1-29, *See* Table 1).

Thus, the presently claimed DYE moiety has been defined functionally with additional physical and structural limitations. Numerous known and unknown DYE moieties when covalently attached to the present crown ethers form chromogenic or fluorogenic ion indicators. The DYE moiety is not intended to be limiting, as many different moieties will work with the present invention. Therefore, Claim 1 read in view of the specification clearly defines what is a DYE moiety. Applicants respectfully request that this rejection of Claim 1 in view of DYE moiety be withdrawn.

Claim 8 as originally filed further limits DYE to benzofuran. Therefore, Claim 8 is not indefinite and Applicants respectfully request that this rejection of Claim 8 be withdrawn.

Newly added Claims 52 and 55 further limit DYE to be "indole, a coumarin, a stilbene, a xanthene, an oxazine, a polyazaindacene, a benzofuran, a pyrene, an

anthracene, a naphthalene, an acridine, a benzindole, an oxazole, a benzoxazole, a thiazole, a benzothiazole, a 4-amino-7-nitrobenz-2-oxa-1,3-diazole (NBD), a cyanine, a carbocyanine, a carbostyryl, a porphyrin, a salicylate, an anthranilate, an azulene, a perylene, a pyridine, a quinoline, a benzoxazine, a carbazine a phenalenone and a benzphenalenone".

Claim 16 has been amended to include the same language for DYE. All other subsequent dependent claims depend from Claims 16, 52 and 55. Therefore, DYE is no longer open ended and Applicants respectfully request that the Examiner withdraw this rejection of Claims 2-21.

Claim 42 specifically limits DYE to polyazaindacene, oxazine or xanthene and Claim 43 claims specific DYEs that are represented by drawn structures. Thus, the Examiner is respectfully requested to withdraw this rejection of Claims 42 and 43.

iii) It is unclear what "a biological compatible esterifying group" or "a biological compatible salt" is.

Claims 1, 21, 42 and 44 have been amended to replace the R^{16} substituent "biologically compatible esterifying group" with alpha-acyloxyalkyl and t-butyldimethylsilyl, which are specific ester forming groups. Applicants believe these claims are now clear and request that the rejection of Claims 1-21, 42 and 43 in view of the claim term "a biologically compatible esterifying group" be withdrawn.

Applicants respectfully traverse the rejection in view of "biologically compatible salt" because the specification provides examples, See Compounds 133, 171 and 177, and the term is a well-known term to those skilled in the art. The present invention permits R^{16} to be a biologically compatible salt, thus any salt ion that is compatible with biological systems may be substituted at the R^{16} position.

Applicants respectfully request that this rejection of Claims 1-21, 42 and 43 be withdrawn in view of the claim term "biologically compatible salt".

iv) The groups at Rx are unclear. These groups are not radicals but compounds or classes of compounds. Appropriate correction is required.

Applicants respectfully traverse this rejection because the reactive groups (Rx) are clear.

A chemically reactive group is a term well known in the art (*See*, R. Haugland, MOLECULAR PROBES HANDBOOK OF FLUORESCENT PROBES AND

REASEARCH CHEMICALS, Chapters 1-3 (1996)). Such a group generally represents a point of attachment for another substance and means a group that is capable of reacting with another chemical group to form a covalent bond. Some require no other reactants for the reaction to occur, for example, the reaction of a succinimidyl ester of a carboxylic acid with an amine. However, some reactive groups, such as carboxylic acid, do typically require activation before a reaction will take place that forms a covalent bond (*See*, the note on the bottom of table 2 on page 28) with another substance to form a dye-conjugate of the present invention. Not all chemical groups are capable of readily reacting with another chemical group. In fact, many chemical groups are inert under many reaction conditions. Because of these reactive group properties, and because these are common terms used in the art, it is not necessary, or feasible, to define all the groups, that are *not* reactive groups but rather it is more important to define *what* constitutes a reactive group.

The present application defines "reactive group" to be a group that facilitates coupling of a present crown ether compound to "a wide variety of organic or inorganic substances that contain or are modified to contain functional groups with suitable reactivity, resulting in chemical attachment of the conjugated substance (Sc)" (page 26 lines 8-9). These moieties capable of chemically reacting with a group on another compound are specifically disclosed in the specification to constitute three species: 1) a nucleophile (page 26, line 11), 2) an electrophile (page 26 line 11), or 3) a photoactivatable group (page 26 line 12). An appropriate nucleophile and electrophile will spontaneously react with each other to form a covalent linkage and a photoactivatable group will react with an appropriate group on another compound when activated with an appropriate wavelength to form a covalent bond.

A nucleophile is a term well known in the art and is generally understood to mean a negative ion or a molecule that has an unshared pair of electrons. More simply stated, nucleophiles are electron-pair donors. Furthermore, it is understood in the art that during a chemical reaction a nucleophile attacks an electron deficient center of another molecule or a positive ion, i.e. an electrophile. An electrophile is also a term well known in the art and is generally understood to mean a positive ion or an electron-accepting group. The present specification provides an extensive table of nucleophiles and

electrophiles (reactive groups) that will react with each other and the resulting covalent bonds that are formed (See, Table 2 page 26-28).

Therefore, Applicants respectfully assert that the specification properly defines the claim term "reactive group". Furthermore, the independent Claims 1, 16 and 21 specifically claim the reactive groups; an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a silyl halide, a sulfonyl halide, or a thiol.

Thus, Applicants respectfully assert that reactive group is not indefinite based on the definition provided in the specification and the limitations found in Claims 1, 16 and 21. Applicants respectfully request that the Examiner withdraw this rejection of Claims 1-21.

Also, language such as "a reactive group selected from the group consisting of" in place of "that is" is suggested.

Claim 1 has been amended as suggested by the Examiner.

v) The metes and bounds of Sc as "a conjugated substance" is unclear.

The present specification discloses "compounds incorporating a reactive group (R_x) can be coupled to a wide variety of organic and inorganic substances that contain or are modified to contain functional groups with suitable reactivity, resulting in chemical attachment of the conjugated substance (S_c)" (page 26 lines 7-10). Thus, a conjugated substance is broadly defined to mean an organic or inorganic substance that contains a reactive group wherein the reactive group will form a covalent bond with the *present compounds* that contain an appropriate reactive group. Inorganic and organic substances are terms well known in the art and reactive group is defined by the present specification. It is generally understood that a chemical reaction between a nucleophile and an electrophile results in a covalent bond; thus, Applicants respectfully assert that this claim term, "conjugated substance", is not considered ambiguous.

The present specification provides numerous preferred embodiments of conjugated substances (page 30 line 23 to page 31 line 31). Many of these preferred embodiments are specifically claimed in the claims (*See*, Claims 13, 14, 16, 33, 52 and 54). These claims clearly define the metes and bounds of a conjugated substance. The Examiner is respectfully requested to withdraw this rejection of Claims 1-21, 42 and 43 in view of the claim term "conjugated substance".

Applicants respectfully request that the Examiner withdraw this rejection of Claims 1-21, 42 and 43 based on the Claim terms "heteroaryl", "DYE", "a biological compatible esterifying group", "a biological compatible salt", "Rx" and "conjugated substance" because 1) one of ordinary skill would know what was intended by these claim terms, 2) these claim terms are adequately defined by the present specification and 3) the terms are commonly used in analogous art references. Furthermore, many of the claims contain preferred reactive groups, conjugated substances and DYE moieties.

CONCLUSION

In view of the above amendments and remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (541) 335-0203.

Respectfully submitted,

Date: February 19, 2004

Koren J. Anderson

Koren J. Anderson, Ph.D.
Reg. No. 51,061

Molecular Probes, Inc.
29851 Willow Creek Rd.
Eugene, Oregon, 97402
Phone: (541) 335-0203
Facsimile: (541) 335-0188

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: MARTIN *et al.*

Serial No.: 10/026,302

Filed: December 19, 2001

For: **Crown Ether Derivatives**

Examiner: Bruck Kifle

Group Art Unit: 1624

MARKED-UP VERSION OF THE CLAIMS

Commissioner for Patents
U.S. Patent and Trademark Office
PO Box 1450
Alexandria, VA 22313-1450

Dear Sir:

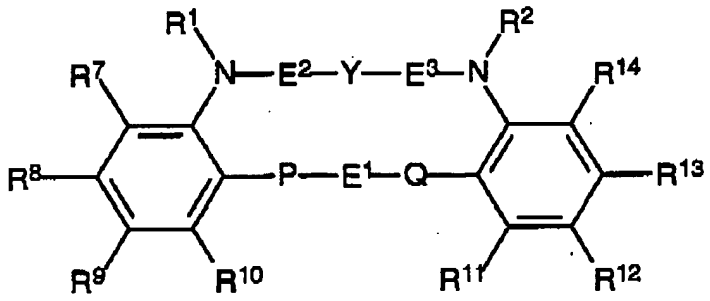
This Marked-up Version of the Claims is being submitted along with the Response to the Office Action dated August 19, 2003. These Marked-up Claims are being submitted on or before the three (3) month extended due date of February 19, 2004. A Petition for Extension of Time is also enclosed (*See*, Transmittal).

The Examiner is respectfully requested to enter the following Claim amendments.

CERTIFICATE OF TRANSMISSION

I HEREBY CERTIFY THAT THIS PAPER AND THE DOCUMENTS REFERRED AS BEING ATTACHED OR ENCLOSED HEREWITH ARE BEING FACSIMILE TRANSMITTED TO THE UNITED STATES PATENT AND TRADMARK OFFICE ON 2/19/04 TO 1.703.872.9306 By Michael A. Sennett

1. (Currently Amended) A compound of the formula



wherein

P and Q are independently O, S, or NR^3, where each R^3 is independently H or C_1-C_6 alkyl;

Y is O, S, or NR^4, where R^4 is H; or is -L-R_X, -L-S_C, or -L-DYE; or is C_1-C_{18} alkyl or an aryl or heteroaryl ring system, which alkyl or ring system is optionally substituted by halogen, azido, nitro, nitroso, amino, C_1-C_6 alkylamino, C_2-C_{12} dialkylamino, cyano, -L--R_X, -L-S_C, or -L-DYE; or by C_1-C_6 alkyl or C_1-C_6 alkoxy that is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$; wherein

R^{15} is H or C_1-C_6 alkyl; or -L-R_X, -L-S_C, or -L-DYE;

R^{16} is H, a C_1-C_6 alkyl, a benzyl, alpha-acyloxyalkyl or t-butyldimethylsilyl ~~a biologically compatible esterifying group~~, a biologically compatible salt; or -L-R_X, -L-S_C, or -L-DYE;

R^{17} and R^{18} are independently H, C_1-C_6 alkyl, C_1-C_8 carboxyalkyl, an alpha-acyloxyalkyl, a t-butyldimethylsilyl, or a biologically compatible salt; or -L-R_X, -L--S_C, or -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;

each L is independently a covalent linkage;

each R_X is independently a reactive group [that is] selected from the group consisting of an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a silyl halide, a sulfonyl halide, or a thiol;

each S_C is independently a conjugated substance;

DYE is a chemical moiety with an absorption maximum beyond 320 nm;

E^1, E^2, and E^3 are independently $-(CR^5_2)_n-$, or $-(C(O)CH_2)_n-$, where n = 2, 3 or 4, and each R^5 is independently H or CH_3, or two R^5 moieties on adjacent carbons of one or more of E^1, E^2 or E^3, when taken in combination, form a 5- or 6-membered aliphatic ring;

R^1 and R^2 are independently -L-R_X, -L-S_C, or -L-DYE; or C_1-C_{18} alkyl or C_7-C_{18} arylalkyl, each of which is optionally substituted by halogen, azido, nitro, nitroso, amino, hydroxy, cyano, or by an aryl or heteroaryl ring system; or by $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, $-(C=O)-NR^{17}R^{18}$; or by C_1-C_6 alkylamino, C_2-C_{12} dialkylamino; or by C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, $-(C=O)-NR^{17}R^{18}$;

R^7-R^{14} are independently H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE; or C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, or $-(C=O)-NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{14}, taken in combination, form a fused six-membered benzo moiety, which is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, or -L-DYE; or C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is optionally substituted by halogen, amino, hydroxy, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, or $-(C=O)-NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{14}, taken in combination with each other, and with the aromatic ring they are bound to, form a fused DYE;

provided that said compound is substituted by at least one -L-DYE, -L-R_X, or -L-S_C at R^1, R^2, R^4, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, or R^{14}; or at least two of R^7-R^{14}, taken in combination, form a fused DYE.

2. (Cancelled) A compound, as claimed in Claim 1, wherein each R^5 is H and each n is 2.

3. (Currently Amended) A compound, as claimed in Claim [1] 52, wherein Y is NR^4.

4. (Cancelled) A compound, as claimed in Claim 1 wherein P and Q are O.

5. (Currently Amended) A compound, as claimed in Claim [4] 52, wherein Y is O.

6. (Original) A compound, as claimed in Claim 5, wherein said compound is substituted by only one -L-R_X, or -L-S_C, that is bound at R^8, R^9, R^{12}, or R^{13}.

7. (Currently Amended) A compound, as claimed in Claim [1] 5, wherein R^1 and R^2 are C_1-C_6 alkyl that are substituted one or more times by cyano, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$.

8. (Original) A compound, as claimed in Claim 1, wherein R^8 and R^9, and optionally R^{12} and R^{13}, taken in combination, form a fused DYE that is a substituted or unsubstituted benzofuran.

9. (Original) A compound, as claimed in Claim 1, wherein said compound is substituted by exactly two DYE or fused DYE moieties.

10. (Currently Amended) A compound, as claimed in Claim [1] 7, wherein said compound is substituted by exactly one -L-DYE moiety at R^9, and said compound is optionally substituted at a position other than R^9 by exactly one -L-R_X or exactly one -L-S_C.

11. (Cancelled) A compound, as claimed in Claim 1, wherein each L is independently a single covalent bond, or a covalent linkage that is linear or branched, cyclic or

heterocyclic, saturated or unsaturated, having 1-20 nonhydrogen atoms selected from the group consisting of C, N, P, O and S; and are composed of any combination of ether, thioether, amine, ester, carboxamide, sulfonamide, hydrazide bonds and aromatic or heteroaromatic bonds.

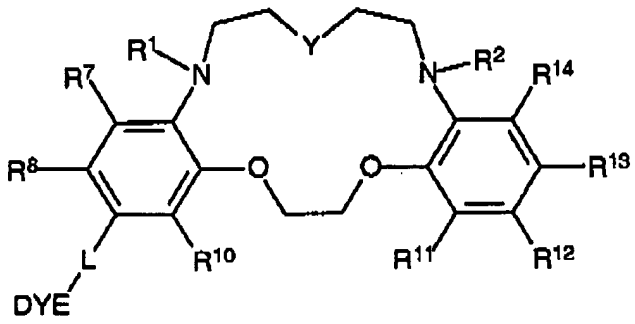
12. (Currently Amended) A compound, as claimed in Claim [11] 54, wherein L is a single covalent bond or has the formula $-(CH_2)_d(CONH(CH_2)_e)_z-$ or $-O(CH_2)_d(CONH(CH_2)_e)_z-$, where d is an integer from 0-5, e is an integer from 1-5 and z is 0 or 1.

13. (Cancelled) A compound, as claimed in Claim 1, that is substituted by at least one S_c selected from the group consisting of an amino acid, a peptide, a protein, a polysaccharide, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell, a biotin, a silica and a virus.

14. (Currently Amended) A compound, as claimed in Claim [13] 6, wherein said compound is substituted by exactly one S_c that is a protein, a polysaccharide, a biotin, a synthetic polymer or a silica.

15. (Currently Amended) A compound, as claimed in Claim [1] 6, that is substituted by at least one $-L-R_x$ selected from the group consisting of a succinimidyl ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide, an aliphatic amine, a silyl halide, and a psoralen.

16. (Currently Amended) A compound[, as claimed in Claim 1,] having the formula



wherein Y is O or NR^4 where R^4 is H; or is -L-R_X, -L-S_C, or -L-DYE; or is C_1-C_{18} alkyl or an aryl or heteroaryl ring system, which alkyl or ring system is optionally substituted by halogen, azido, nitro, nitroso, amino, C_1-C_6 alkylamino, C_2-C_{12} dialkylamino, cyano, -L-R_X, -L-S_C, or -L-DYE; or by C_1-C_6 alkyl or C_1-C_6 alkoxy that is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{15}, or -(C=O)-$NR^{17}R^{18}$; wherein

R^{15} is H or C_1-C_6 alkyl; or -L-R_X, -L-S_C, or -L-DYE;

R^{16} is H, a C_1-C_6 alkyl, a benzyl, alpha-acyloxyalkyl, t-butyldimethylsilyl, a biologically compatible salt; or -L-R_X, -L-S_C, or -L-DYE;

R^{17} and R^{18} are independently H, C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, an alpha-acyloxyalkyl, a t-butyldimethylsilyl, or a biologically compatible salt; or -L-R_X, -L-S_C, or -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;

each L is independently a covalent linkage;

each R_X is independently a reactive group selected from the group consisting of an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a silyl halide, a sulfonyl halide, or a thiol;

each S_C is selected from the group consisting of an amino acid, a peptide, a protein, a polysaccharide, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell, a biotin, a silica and a virus;

DYE is selected from the group consisting of indole, a coumarin, a stilbene, a xanthene, an oxazine, a polyazaindacene, a benzofuran, a pyrene, an anthracene, a naphthalene, an acridine, a benzindole, an oxazole, a

benzoxazole, a thiazole, a benzothiazole, a 4-amino-7-nitrobenz-2-oxa-1,3-diazole (NBD), a cyanine, a carbocyanine, a carbostyryl, a porphyrin, a salicylate, an anthranilate, an azulene, a perylene, a pyridine, a quinoline, a benzoxazine, a carbazine a phenalenone and a benzphenalenone;

R^1 and R^2 are independently -L-R_X, -L-S_C, or -L-DYE; or C_1-C_{18} alkyl or C_7-C_{18} arylalkyl, each of which is optionally substituted by halogen, azido, nitro, nitroso, amino, hydroxy, cyano, or by an aryl or heteroaryl ring system; or by -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, -(C=O)-$NR^{17}R^{18}$; or by C_1-C_6 alkylamino, C_2-C_{12} dialkylamino; or by C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, -(C=O)-$NR^{17}R^{18}$; and,

R^7, R^9 and R^{10}-R^{14} are independently H, halogen, azido, nitro, nitroso, amino, cyano, -L--R_X, -L-S_C, -L-DYE; or C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$,

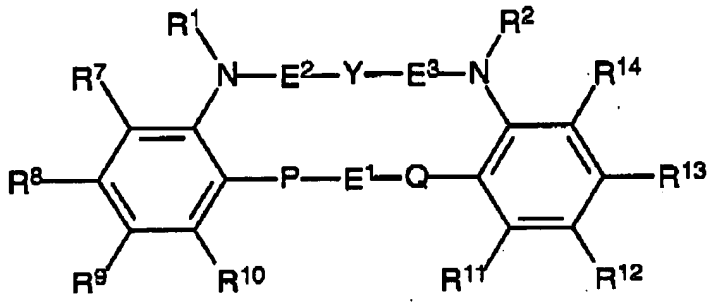
17. (Original) A compound, as claimed in Claim 16, wherein DYE is an indole, a coumarin, a stilbene, a xanthene, an oxazine, or a polyazaindacene.

18. (Currently Amended) A compound, as claimed in Claim 17, wherein ~~DYE~~ xanthene is selected from the group consisting of a fluorescein, a rhodamine, a rhodol, a ~~polyazaindacene, an oxazine,~~ a 3*H*-xanthen-6-ol-3-one, a 6-amino-3*H*-xanthen-3-one, ~~or~~ and a 6-amino-3*H*-xanthen-3-imine[,]; and L is a single covalent bond.

19. (Currently Amended) A compound, as claimed in Claim [16] 18, wherein R^1 and R^2 are C_1-C_6 alkyl that are substituted one or more times by -(C=O)-O-R^{16} or -(C=O)-$NR^{17}R^{18}$.

20. (Original) A compound, as claimed in Claim 19, wherein R^1 and R^2 are C_1-C_6 alkyl that are substituted one or more times by -(C=O)-O-R^{16}, where each R^{16} is H, C_1-C_6 alkyl, an alpha-acyloxymethyl, a t-butyldimethyldimethylsilyl, or a biologically compatible salt.

21. (Currently Amended) A composition of matter comprising a compound of the formula:



wherein

P and Q are independently O, S, or NR^3, where each R^3 is independently H or C_1-C_6 alkyl;

Y is O, S, or NR^4, where R^4 is H; or is -L-R_X, -L-S_C, or -L-DYE; or is C_1-C_{18} alkyl or an aryl or heteroaryl ring system, which alkyl or ring system is optionally substituted by halogen, azido, nitro, nitroso, amino, C_1-C_6 alkylamino, C_2-C_{12} dialkylamino, cyano, -L-R_X, -L-S_C, or -L-DYE; or by C_1-C_6 alkyl or C_1-C_6 alkoxy that is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$; wherein

R^{15} is H or C_1-C_6 alkyl; or -L-R_X, -L-S_C, or -L-DYE;

R^{16} is H, a C_1-C_6 alkyl, a benzyl, alpha-acyloxyalkyl, t-butyldimethylsilyl ~~a biologically compatible esterifying group~~, a biologically compatible salt; or -L-R_X, -L-S_C, or -L-DYE;

R^{17} and R^{18} are independently H, C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, an alpha-acyloxyalkyl, a t-butyldimethylsilyl, or a biologically compatible salt; or -L-R_X, -L-S_C, or -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;

each L is independently a covalent linkage;

each R_X is independently a reactive group selected from the group consisting of an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a silyl halide, a sulfonyl halide, or a thiol;

each S_C is independently a conjugated substance;

DYE is a chemical moiety with an absorption maximum beyond 320 nm;

E^1, E^2, and E^3 are independently $-(CR^5_2)_n-$, where n = 2, 3, or 4, and each R^5 is independently H or CH_3, or two R^5 moieties on adjacent carbons of one or more of E^1, E^2 or E^3, when taken in combination, form a 5- or 6-membered aliphatic ring;

R^1 and R^2 are independently -L-R_X, -L-S_C, or -L-DYE; or C_1-C_{18} alkyl or C_7-C_{18} arylalkyl, each of which is optionally substituted by halogen, azido, nitro, nitroso, amino, hydroxy, cyano, or by an aryl or heteroaryl ring system; or by $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, $-(C=O)-NR^{17}R^{18}$; or by C_1-C_6 alkylamino, C_2-C_{12} dialkylamino; or by C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, $-(C=O)-NR^{17}R^{18}$;

R^7-R^{14} are independently H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE; or C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, or $-(C=O)-NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{14}, taken in combination, form a fused six-membered benzo moiety, which is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, or -L-DYE; or C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is optionally substituted by halogen, amino, hydroxy, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, or $-(C=O)-NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{14}, taken in combination with each other, and with the aromatic ring they are bound to, form a fused DYE;

provided that said compound is substituted by at least one -L-DYE moiety at one or more of R^1, R^2, R^3, and R^7-R^{14}; or at least two of R^7-R^{14}, taken in combination, form a fused DYE.

22. (Cancelled) A composition, as claimed in Claim 21, wherein each R^5 of the compound is H and each n is 2.

23. (Cancelled) A composition, as claimed in Claim 21, wherein each R_x of the compound is independently a reactive group that is an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a silyl halide, a sulfonyl halide, or a thiol.

24. (Cancelled) A composition, as claimed in Claim 21, wherein each P and Q are O.

25. (Currently Amended) A composition, as claimed in Claim [24] 55, wherein each Y is O.

26. (Original) A composition, as claimed in Claim 25, wherein said compound is substituted by only one -L-R_x, or -L-S_c, that is bound at R^8, R^9, R^{12}, or R^{13}.

27. (Original) A composition, as claimed in Claim 25, wherein R^1 and R^2 are C_1-C_6 alkyl that are substituted one or more times by cyano, -(C=O)-O-R^{16}, or -(C=O)-N$R^{17}R^{18}$.

28. (Currently Amended) A composition, as claimed in Claim [21] 27, wherein R^8 and R^9, and optionally R^{12} and R^{13}, taken in combination, form a fused DYE that is a substituted or unsubstituted benzofuran.

29. (Currently Amended) A composition, as claimed in Claim [21] 25, wherein said compound is substituted by exactly two DYE or fused DYE moieties.

30. (Currently Amended) A composition, as claimed in Claim [21] 25, wherein said compound is substituted by exactly one -L-DYE moiety at R^9, and said compound is optionally substituted by exactly one -L-R_X or exactly one -L-S_C at a position other than R^9.

31. (Original) A composition, as claimed in Claim 21, wherein each L of the compound is independently a single covalent bond, or a covalent linkage that is linear or branched, cyclic or heterocyclic, saturated or unsaturated, having 1-20 nonhydrogen atoms selected from the group consisting of C, N, P, O and S; and are composed of any combination of ether, thioether, amine, ester, carboxamide, sulfonamide, hydrazide bonds and aromatic or heteroaromatic bonds.

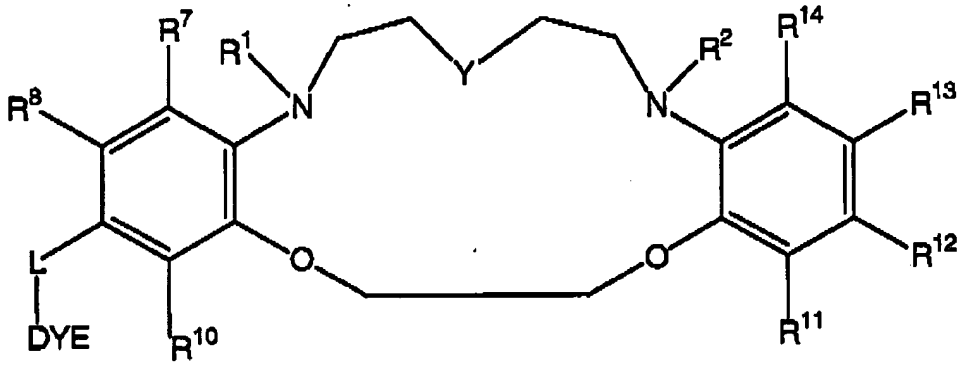
32. (Original) A composition, as claimed in Claim 31, wherein each L of the compound is a single covalent bond or has the formula -$(CH_2)_d(CONH(CH_2)_e)_z$- or -$O(CH_2)_d(CONH(CH_2)_e)_z$-, where d is an integer from 0-5, e is an integer from 1-5 and z is 0 or 1.

33. (Cancelled) A composition, as claimed in Claim 21, wherein each S_C of the compound is an amino acid, a peptide, a protein, a polysaccharide, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell, a biotin, a silica or a virus.

34. (Currently Amended) A composition, as claimed in Claim [33] 26, wherein said compound is substituted by exactly one S_C, which S_C is a protein, a polysaccharide, a biotin, or a silica.

35. (Currently Amended) A composition, as claimed in Claim [21] 26, wherein said compound is substituted by exactly one R_X selected from the group consisting of a succinimidyl ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide, an aliphatic amine, a silyl halide, and a psoralen.

36. (Previously Amended) A composition, as claimed in Claim 21, where the compound has the formula:



wherein Y is O or NR^4.

37. (Original) A composition, as claimed in Claim 36, wherein each DYE on the compound is a fluorescein, a rhodamine, a rhodol, a polyazaindacene, an oxazine, a 3*H*-xanthen-6-ol-3-one, a 6-amino-3*H*-xanthen-3-one, or a 6-amino-3*H*-xanthen-3-imine.

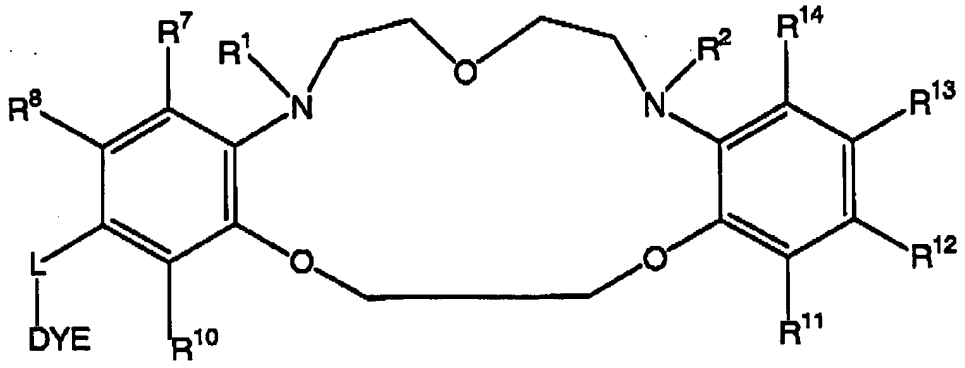
38. (Currently Amended) A composition, as claimed in Claim [36] 37, wherein R^1 and R^2 are C_1-C_6 alkyl that are substituted one or more times by -(C=O)-O-R^{16} or -(C=O)-$NR^{17}R^{18}$.

39. (Original) A composition, as claimed in Claim 38, wherein R^1 and R^2 are C_1-C_6 alkyl that are substituted one or more times by -(C=O)-O-R^{16}, where each R^{16} is H, an alpha-acyloxymethyl, a t-butyldimethyldimethylsilyl, or a biologically compatible salt.

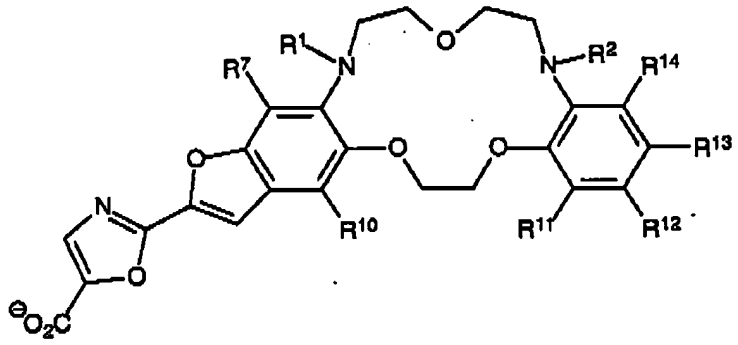
40. (Original) A composition, as claimed in Claim 36, further comprising a metal ion that is Ca^{2+}, Na^+, K^+, or Zn^{2+} associated with said compound.

41. (Original) A composition, as claimed in Claim 21, further comprising a natural or synthetic polymer or glass.

42. (Currently Amended) A compound having the formula:



or the formula:



wherein

R^1 and R^2 are C_1-C_6 alkyl that are substituted one or more times by cyano, an aryl or heteroaryl ring system, or by -(C=O)-O-R^{16} or -(C=O)-$NR^{17}R^{18}$, where

R^{16} is H, a C_1-C_6 alkyl, a benzyl, alpha-acyloxyalkyl, t-butyldimethylsilyl ~~a biologically compatible esterifying group~~, or a biologically compatible salt;

R^{17} and R^{18} are independently H, C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, an alpha-acyloxymethyl, or a biologically compatible salt;

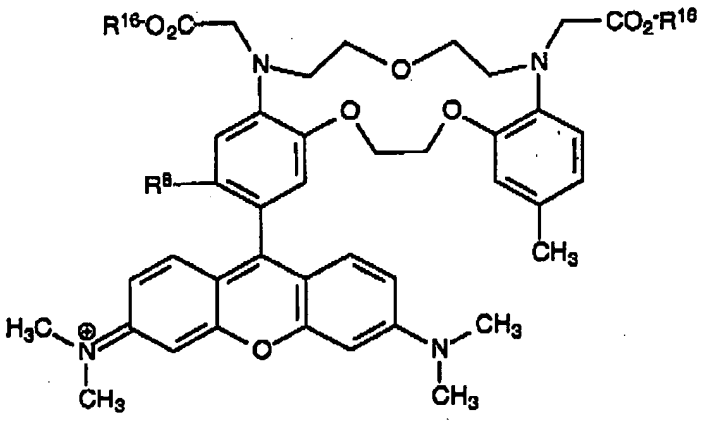
R^7-R^{10}, and R^{11}-R^{14}, where present, are independently H, chloro, bromo, fluoro, nitro, amino, or cyano; or C_1-C_6 alkyl or C_1-C_6 alkoxy that is itself optionally

substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

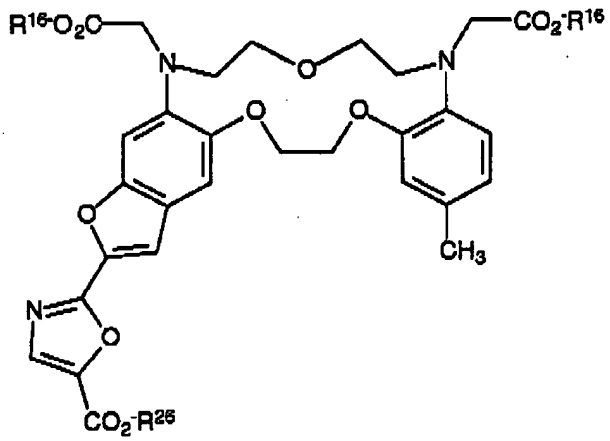
L is a covalent linkage; and

DYE, where present is a polyazaindacene, an oxazine, or a xanthene, which is optionally substituted by halogen, nitro, sulfo, cyano, an aryl or heteroaryl ring system, or benzo, or alkyl, perfluoroalkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, arylalkyl, or acyl, the alkyl portions of which contain fewer than 20 carbons.

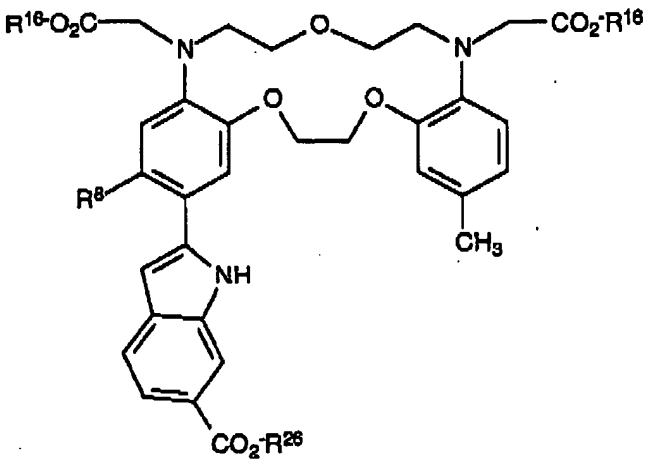
43. (Currently Amended) A compound having the formula:



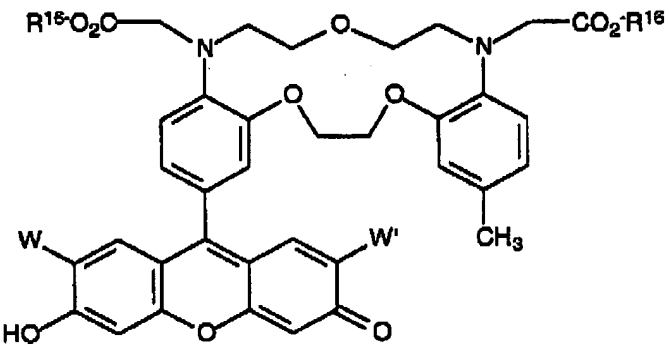
or the formula



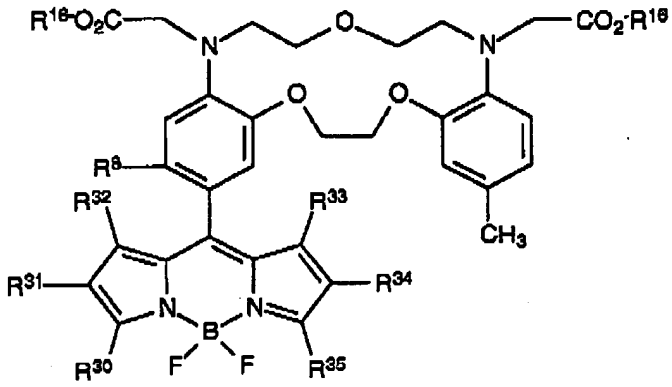
or the formula:



or the formula:



or the formula:



wherein

R^8, where present, is independently H or a C_1-C_6 ~~alkyoxy~~ alkoxy, which is optionally substituted by -(C=O)-O-R^{16} or -(C=O)-$NR^{17}R^{18}$;

R^{16} and R^{26}, where present, are independently H, a C_1-C_6 alkyl, a benzyl, an alpha-acyloxyalkyl, a t-butyldimethylsilyl, or a biologically compatible salt;

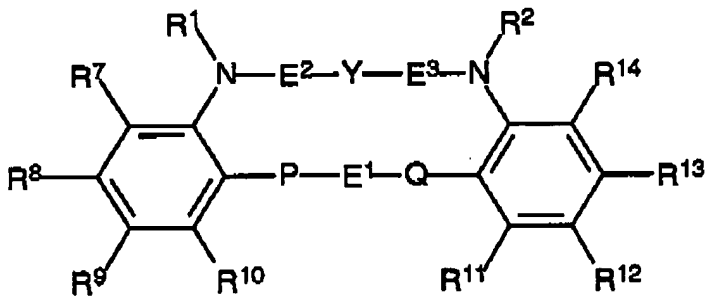
R^{17} and R^{18}, where present, are independently H, a C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, or a biologically compatible salt;

W and W', where present, are independently F or Cl;

R^{30}-R^{35}, where present, are independently H, halogen, nitro, sulfo, cyano, alkyl, perfluoroalkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, arylalkyl, or acyl, wherein the alkyl portions of each contain fewer than 20 carbons; or an aryl or heteroaryl ring system; or adjacent substituents R^{31} and R^{32}, and adjacent substituents R^{33} and R^{34}, when taken in combination form a fused benzo ring that is optionally substituted by hydrogen, halogen, nitro, sulfo, cyano, alkyl, perfluoroalkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, alkylthio, alkylamido, amino, monoalkylamino or dialkylamino wherein the alkyl portions of each contain fewer than 20 carbons.

44. (Currently Amended) A method of detecting a target cationic metal ion in a sample, comprising:

a) adding to said sample, in an amount sufficient to generate a detectable optical response when said target ion is present, a compound having the formula:



wherein

P and Q are independently O, S, or NR^3, where each R^3 is independently H or C_1-C_6 alkyl;

Y is O, S, or NR^4, where R^4 is H; or is -L-R_X, -L-S_C, or -L-DYE; or is C_1-C_{18} alkyl or an aryl or heteroaryl ring system, which alkyl or ring system is optionally substituted by halogen, azido, nitro, nitroso, amino, C_1-C_6 alkylamino, C_2-C_{12} dialkylamino, cyano, -L-R_X, -L-S_C, or -L-DYE; or by C_1-C_6 alkyl or C_1-C_6 alkoxy that is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$; wherein

R^{15} is H or C_1-C_6 alkyl; or -L-R_X, -L-S_C, or -L-DYE;

R^{16} is H, a C_1-C_6 alkyl, a benzyl, alpha-acyloxyalkyl and t-butyldimethylsilyl a ~~biologically compatible esterifying group~~, a biologically compatible salt; or -L-R_X, -L-S_C, or -L-DYE;

R^{17} and R^{18} are independently H, C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, an alpha-acyloxyalkyl, a t-butyldimethylsilyl, or a biologically compatible salt; or -L-R_X, -L-S_C, or -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;

each L is independently a covalent linkage;

each R_X is independently a reactive group;

each S_C is independently a conjugated substance;

DYE is a chemical moiety with an absorption maximum beyond 320 nm;

E^1, E^2, and E^3 are independently $-(CR^5_2)_n-$, where n = 2, 3, 4, and each R^5 is independently H or CH_3, or two R^5 moieties on adjacent carbons of one or more of E^1, E^2 or E^3, when taken in combination, form a 5- or 6-membered aliphatic ring;

R^1 and R^2 are independently -L-R_X, -L-S_C, or -L-DYE; or C_1-C_{18} alkyl or C_7-C_{18} arylalkyl, each of which is optionally substituted by halogen, azido, nitro, nitroso, amino, hydroxy, cyano, or by an aryl or heteroaryl ring system; or by $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, $-(C=O)-NR^{17}R^{18}$; or by C_1-C_6 alkylamino, C_2-C_{12} dialkylamino; or by C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, $-(C=O)-NR^{17}R^{18}$;

R^7-R^{14} are independently H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE; or C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, or $-(C=O)-NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{14}, taken in combination, form a fused six-membered benzo moiety, which is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, or -L-DYE; or C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is optionally substituted by halogen, amino, hydroxy, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, or $-(C=O)-NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{14}, taken in combination with each other, and with the aromatic ring they are bound to, form a fused DYE;

provided that said compound is substituted by at least one -L-DYE moiety at one or more of R^1, R^2, R^3, and R^7-R^{14}; or at least two of R^7-R^{14}, taken in combination, form a fused DYE;

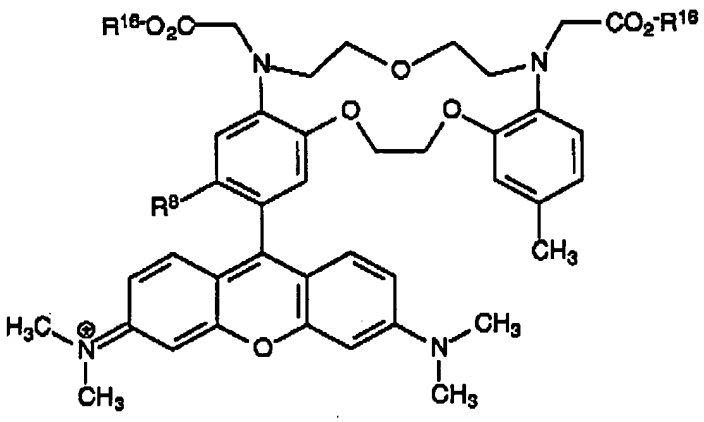
b) illuminating said sample to generate said detectable optical response whereby said target ion is present.

45. (Original) A method, as claimed in Claim 44, wherein said detectable optical response is a fluorescence response.

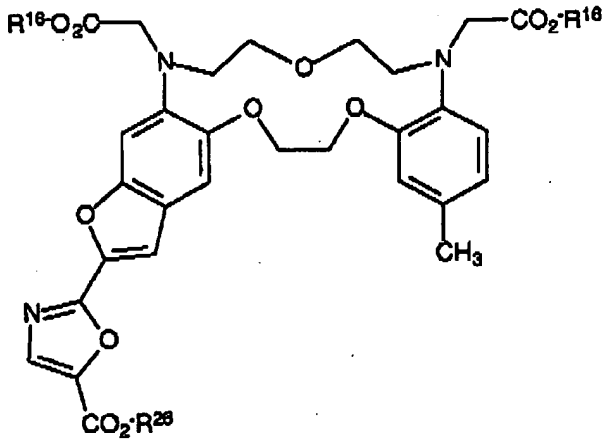
46. (Previously Amended) A method, as claimed in Claim 45, wherein said illuminating is performed in conjunction with a fluorometer, fluorescence microscope, laser scanner, flow cytometer, a microfluidic device, or a fiber optic probe.

47. (Original) A method, as claimed in Claim 44, wherein said target metal ion is Na^+, K^+, Ca^{2+}, or Zn^{2+}.

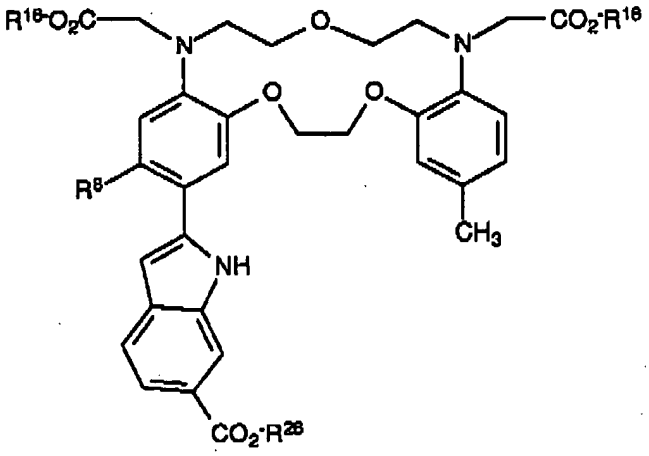
48. (Currently Amended) A method, as claimed in Claim 44, wherein said compound has the formula:



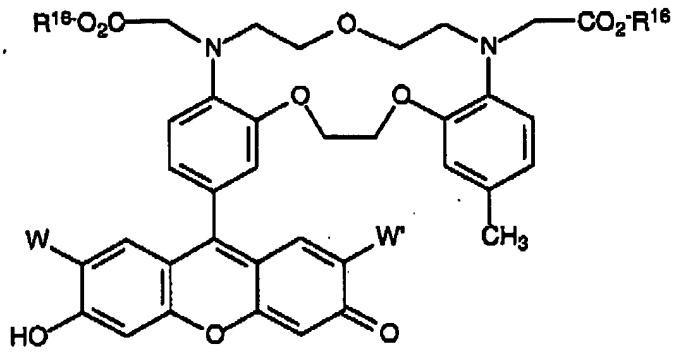
or the formula:



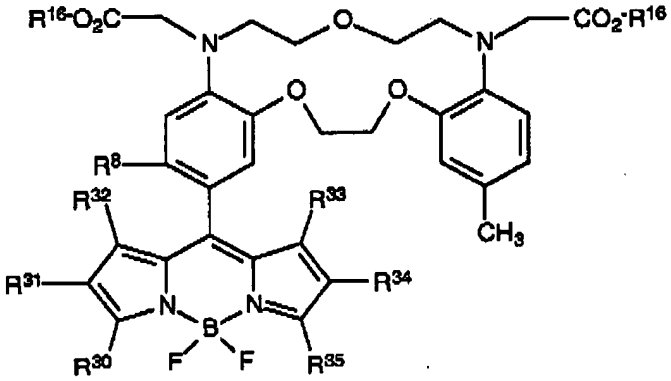
or the formula:



or the formula:



or the formula:



wherein

R^8, where present, is independently H or a C_1-C_6 ~~alkyoxy~~ alkoxy, which is optionally substituted by -(C=O)-O-R^{16} or -(C=O)-$NR^{17}R^{18}$;

R^{16} and R^{25}, where present, are independently H, a C_1-C_6 alkyl, a benzyl, an alpha-acyloxyalkyl, a t-butyldimethylsilyl, or a biologically compatible salt;

R^{17} and R^{18}, where present, are independently H, a C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, or a biologically compatible salt;

W and W', where present, are independently F or Cl;

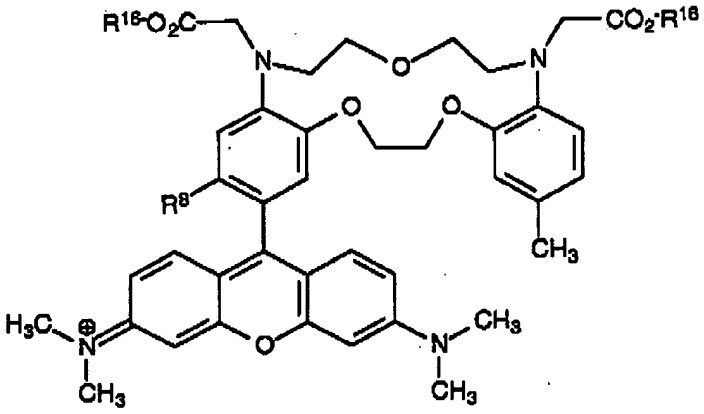
R^{30}-R^{35}, where present, are independently H, halogen, nitro, sulfo, cyano, alkyl, perfluoroalkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, arylalkyl, or acyl, wherein the

alkyl portions of each contain fewer than 20 carbons; or an aryl or heteroaryl ring system.

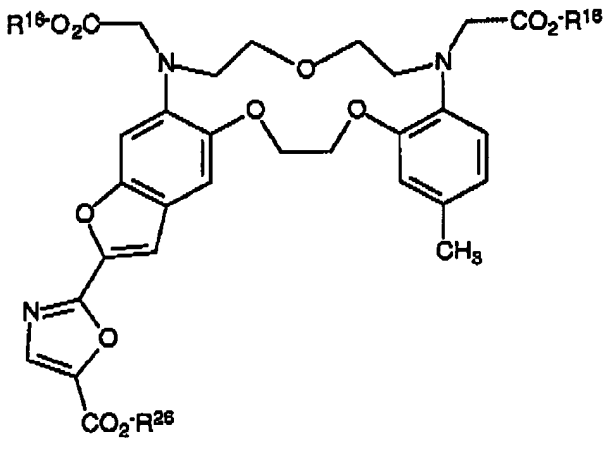
49. (Original) A method, as claimed in Claim 48, wherein said target metal ion is Na^+ or K^+.

50. (Previously Amended) A method, as claimed in Claim 44, wherein said sample comprises living cells or biological fluids.

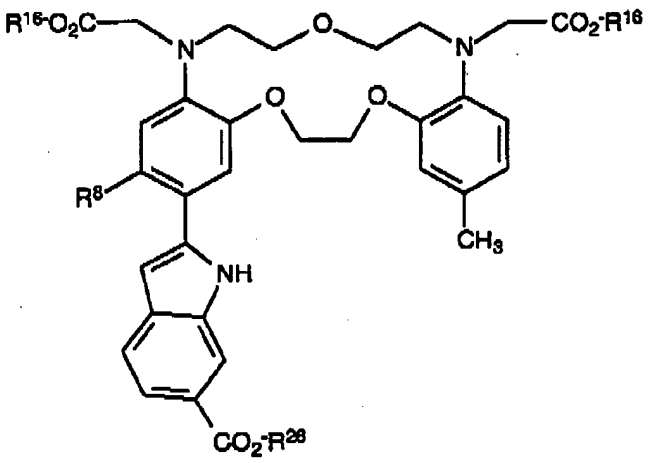
51. (Currently Amended) A kit for the detection or quantification of a target metal ion, comprising a compound having the formula:



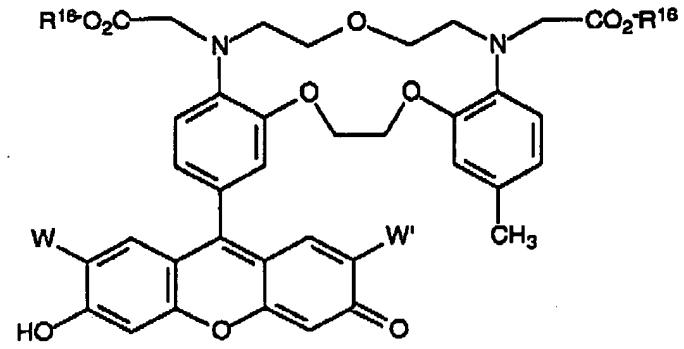
or the formula:



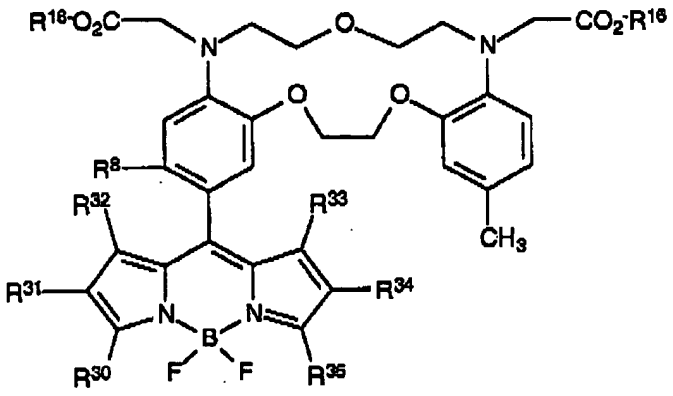
or the formula:



or the formula:



or the formula:



wherein

R^8, where present, is independently H or a C_1-C_6 ~~alkyoxy~~ alkoxy, which is optionally substituted by -(C=O)-O-R^{16} or -(C=O)-$NR^{17}R^{18}$;

R^{16} and R^{26}, where present, are independently H, a C_1-C_6 alkyl, a benzyl, an alpha-acyloxyalkyl, a t-butyldimethylsilyl, or a biologically compatible salt;

R^{17} and R^{18}, where present, are independently H, a C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, or a biologically compatible salt;

W and W', where present, are independently F or Cl;

R^{30}-R^{35}, where present, are independently H, halogen, nitro, sulfo, cyano, alkyl, perfluoroalkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, arylalkyl, or acyl, wherein the alkyl portions of each contain fewer than 20 carbons; or an aryl or heteroaryl ring system;

and comprising one or more components selected from the group consisting of:

a) a calibration standard of a target ion;

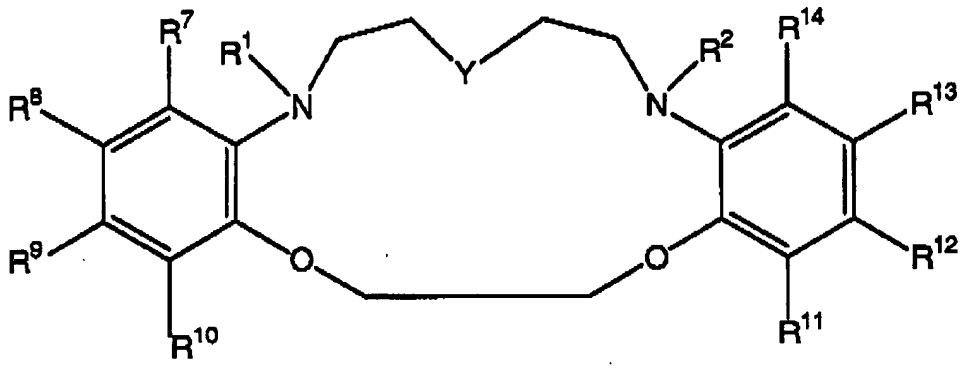
b) an ionophore;

c) a fluorescence standard;

d) an aqueous buffer solution; and

e) an organic solvent.

52. (New) The Compound according to Claim 1, wherein said compound has the formula:



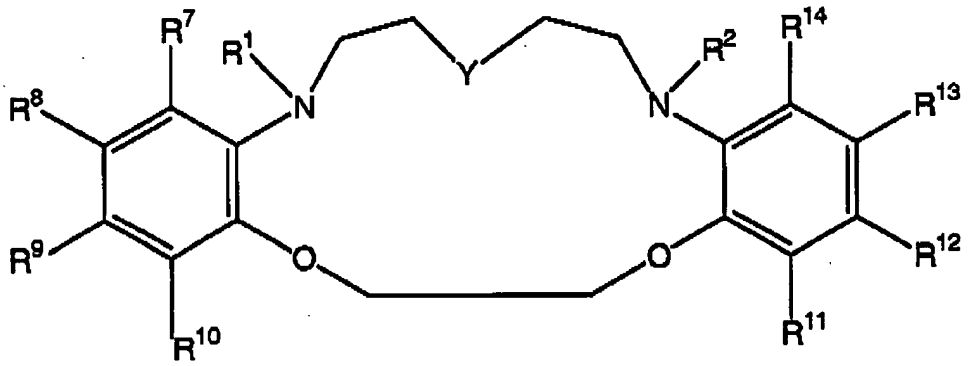
wherein said DYE is selected from the group consisting of indole, a coumarin, a stilbene, a xanthene, an oxazine, a polyazaindacene, a benzofuran, a pyrene, an anthracene, a naphthalene, an acridine, a benzindole, an oxazole, a benzoxazole, a thiazole, a benzothiazole, a 4-amino-7-nitrobenz-2-oxa-1,3-diazole (NBD), a cyanine, a carbocyanine, a carbostyryl, a porphyrin, a salicylate, an anthranilate, an azulene, a perylene, a pyridine, a quinoline, a benzoxazine, a carbazine a phenalenone and a benzphenalenone; and,

each S_C is selected from the group consisting of an amino acid, a peptide, a protein, a polysaccharide, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell, a biotin, a silica and a virus.

53. (New) The compound according to Claim 7, wherein said R^{16}, R^{17} and R^{18} are independently H or C_1-C_6 alkyl.

54. (New) The compound according to Claim 10, wherein said DYE is selected from the group consisting of indole, a coumarin, a stilbene, a xanthene, an oxazine, or a polyazaindacene.

55. (New) The composition according to Claim 21, wherein said compound has the formula:



wherein said DYE is selected from the group consisting of indole, a coumarin, a stilbene, a xanthene, an oxazine, a polyazaindacene, a benzofuran, a pyrene, an anthracene, a naphthalene, an acridine, a benzindole, an oxazole, a benzoxazole, a thiazole, a benzothiazole, a 4-amino-7-nitrobenz-2-oxa-1,3-diazole (NBD), a cyanine, a carbocyanine, a carbostyryl, a porphyrin, a salicylate, an anthranilate, an azulene, a perylene, a pyridine, a quinoline, a benzoxazine, a carbazine a phenalenone and a benzphenalenone; and,

each S_C is selected from the group consisting of an amino acid, a peptide, a protein, a polysaccharide, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell, a biotin, a silica and a virus.

Martin *et al.*
Serial No. 10/026,302

Respectfully submitted,

Date: February 19, 2004

Koren J. Anderson, Ph.D.
Reg. No. 51,061

Molecular Probes, Inc.
29851 Willow Creek Rd.
Eugene, Oregon, 97402
Phone: (541) 335-0203
Facsimile: (541) 335-0188